

CLAIMS

What is claimed is:

1. (Currently amended) A nucleic acid sequence which comprises ~~comprising~~:

$P_x-S_x-B_n-(ZR)$ -transport peptide- (Z_1Z_2) -protein(Y)-T; wherein:

the nucleic acid codes for a fusion protein comprising a peptide encoded by transport peptide linked via a peptide encoded by a first Z_1Z_2 to a protein encoded by said protein(Y) which is linked to T;

the peptide encoded by transport peptide improves the rate of secretion of the protein encoded by said protein(Y);

P_x comprises a promoter sequence;

S_x comprises a nucleic acid sequence encoding a signal or leader sequence;

B_n is a chemical bond;

Z is a codon for lysine or arginine;

R is an arginine codon;

transport peptide comprises a nucleic acid sequence encoding hirudin or an hirudin variant;

Z_1 is a codon for lysine or arginine or a portion thereof or a chemical bond when Z_1 and Z_2 combine to make the second Z_1Z_2 and $m=0$;

Z_2 is a codon for lysine or arginine or a portion thereof or a chemical bond when Z_1 and Z_2 combine to make the second Z_1Z_2 and $m=0$;

protein(Y), ~~selected from the group consisting of mini-proinsulin and proinsulin,~~ comprises a nucleic acid sequence encoding a protein, selected from the group consisting of mini-proinsulin and proinsulin, that is produced and secreted by yeast; and

T is an untranslated expression-enhancing nucleic acid sequence.

Claims 2 - 5. (Canceled)

6. (Original) A multicopy vector comprising the nucleic acid of claim 1.
7. (Original) A plasmid comprising the nucleic acid of claim 1.
8. (Original) A host cell comprising the nucleic acid of claim 1 as a part of the host cell chromosome, as a part of a mini-chromosome, or extra-chromosomally.
9. (Original) The host cell of claim 8, wherein the host cell is a yeast.
10. (Original) The host cell of 9, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*, *Hansenula polymorpha*, and *Pichia pastoris*.
11. (Original) A host cell comprising the multicopy vector of claim 6.
12. (Original) A host cell comprising the plasmid of claim 7.

Claims 13-26. (Canceled)

27. (Previously presented) An engineered host cell comprising the nucleic acid of claim 1 as a part of the host cell chromosome, as a part of a mini-chromosome, or extra-chromosomally.
28. (Previously presented) An engineered host cell comprising the multicopy vector of claim 6.
29. (Previously presented) An engineered host cell comprising the plasmid of claim 7.
30. (New) An article of manufacture comprising a nucleic acid sequence which comprises:

$P_x-S_x-B_n-(ZR)-\text{transport peptide}-(Z_1Z_2)-\text{protein}(Y)-T$; wherein:

the nucleic acid codes for a fusion protein comprising a peptide encoded by transport peptide linked via a peptide encoded by a first Z_1Z_2 to a protein encoded by said protein(Y) which is linked to T;

the peptide encoded by transport peptide improves the rate of secretion of the protein encoded by said protein(Y);

P_x comprises a promoter sequence;

S_x comprises a nucleic acid sequence encoding a signal or leader sequence;

B_n is a chemical bond;

Z is a codon for lysine or arginine;

R is an arginine codon;

transport peptide comprises a nucleic acid sequence encoding hirudin or an hirudin variant;

Z_1 is a codon for lysine or arginine or a portion thereof or a chemical bond when Z_1 and Z_2 combine to make the second Z_1Z_2 and $m=0$;

Z_2 is a codon for lysine or arginine or a portion thereof or a chemical bond when Z_1 and Z_2 combine to make the second Z_1Z_2 and $m=0$;

protein(Y) comprises a nucleic acid sequence encoding a protein, selected from the group consisting of mini-proinsulin and proinsulin, that is produced and secreted by yeast; and

T is an untranslated expression-enhancing nucleic acid sequence.

31. (New) A multicopy vector comprising the nucleic acid of claim 30.

32. (New) A plasmid comprising the nucleic acid of claim 30.

33. (New) A host cell comprising the nucleic acid of claim 30 as a part of the host cell chromosome, as a part of a mini-chromosome, or extra-chromosomally.

34. (New) The host cell of claim 33, wherein the host cell is a yeast.

35. (New) The host cell of 34, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*, *Hansenula polymorpha*, and *Pichia pastoris*.

36. (New) A host cell comprising the multicopy vector of claim 31.

37. (New) A host cell comprising the plasmid of claim 32.